

IMMUNE PRIMING
Immunize mice with imported
fire ant midgut tissue

RNA → cDNA
Isolate total RNA from spleen of immunized mice,
prepare cDNA by reverse transcription, amplify
by polymerase chain reaction, purify cDNA from gel

**ANTIBODY LIBRARY ON
SURFACE OF PHAGE**
Create phage display library
expressing 10^6 - 10^8 unique
antibody Fab fragments

DUAL MIDGUT SELECTION
Two-step absorptions to yield
phage displaying antibody fragments
specific for midgut of imported fire ants
and not native fire ants

FINAL MICROVILLI SELECTION
Immunohistochemical verification of
Fab specific to imported fire ant
microvilli cells

TESTING
Test phage/Fab for internalization by
microvilli cells of imported fire ants
when administered by feeding

IMPORTED FIRE ANT ERADICATION
Test phage/Fab/gelonin Conjugate for ability
to selectively kill imported fire ants.

FIGURE 1

Figure 2 Evaluation of Monoclonal Antibodies to Midgut Antigens of Imported and Native Fire Ant queens.

Immunohistological analyses of monoclonal antibody binding to the midgut antigens of imported fire ant queens (Positive, B) but not to the midgut antigens of midgut antigens from native fire ants (Native, C). Midgut antigens from imported fire ants reacted with irrelevant antibody did not stain positive and served as the negative control (Control, A)

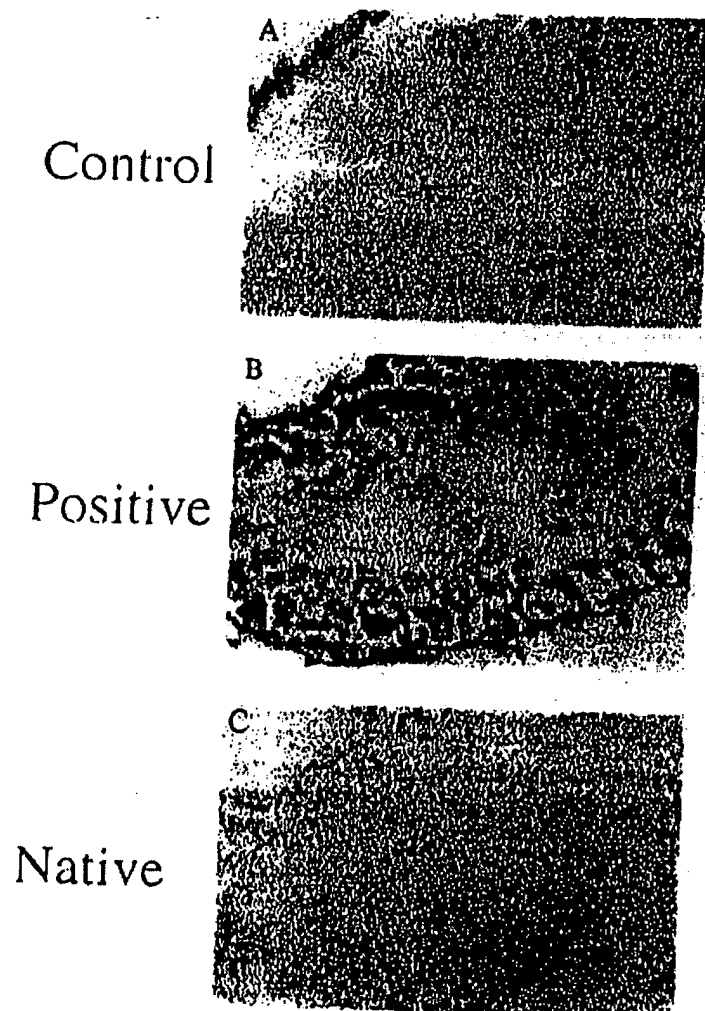


Fig. 2

Figure 2, Purification of Phage Displayed Fab to Midgut Antigens of Imported Fire Ant Queens.

EVIDENCE OF PRESENCE OF IG FAB FRAGMENT

C = Control

1-4 = eluted soluble Fab (sFab)

46 = size of Fab fragment (46 Kb)

Western immunoblot analyses show the clone to express Ig Fab. These clones were selected for ability to bind to antigens of the midgut of imported fire ants but not to the midgut antigens of native fire ants.

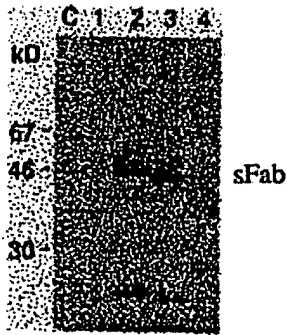


Fig. 3

Figure 4. Purification of Phage Displayed Fab to Gelonin

EVIDENCE OF PRESENCE OF IG FAB FRAGMENT FOR TWO CLONES: (pComb3/Fab (6) and pComb3/Fab (47).

IN = Induced

U = Uninduced

V = bacteria containing virus without Ig Fab

46 Kb = size of Fab fragment

Western immunoblot analyses show that clones pComb3/Fab (6) and pComb3/Fab (47) express Ig Fab. These clones were selected for ability to bind to gelonin.

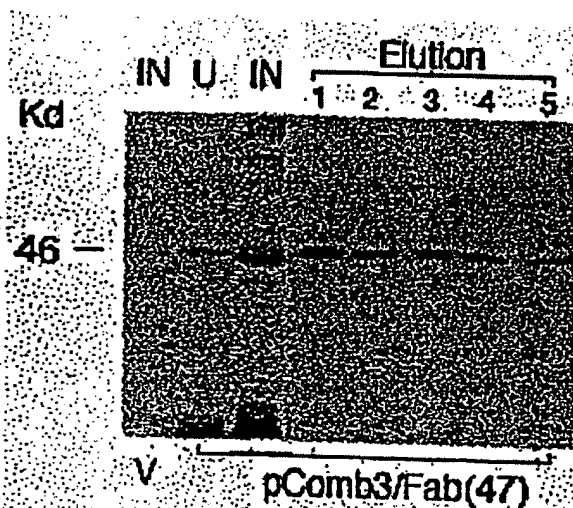
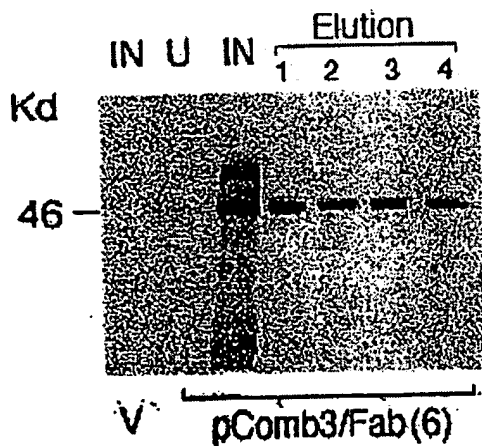


Fig. 4